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Abstract

The importance of horizontal gene transfer (HGT) in bacterial evolution is evident from the retrospective analyses of bacterial genomes, which suggest that a substantial part of bacterial genomes is of foreign origin. Another line of evidence that supports the possibility of rapid adaptation of bacteria through lateral gene exchange is the history of antibiotic use by humans. Within a very brief period of the 'antibiotic era' many bacterial pathogens were able to acquire the mechanisms allowing them to withstand the selective pressure of antibiotics. And, finally, the field and microcosm studies allowed monitoring HGT events *in situ*. In this chapter, a brief overview of the milestones of mobile genetic elements (MGEs) research is given, followed by discussion of the conceptual framework development. Then the occurrence and diversity of MGEs as well as the frequencies of HGT in terrestrial, aquatic, intestinal and biofilm communities are described. The role of environmental factors that may affect MGE-mediated HGT in these ecosystems is also discussed.

Introduction

The first horizontal gene transfer experiments were performed by Griffith (1928), who 'transformed' rough, avirulent live pneumococci into smooth, virulent pneumococci by adding factors from dead, smooth and virulent pneumococci. The word 'transformation' at his time had no genetic connotation in the sense we would perceive it today; it was a 'transformation' of the pneumococcal phenotype. The nature of the transforming factor remained unknown until the 1940s, when it was recognized as DNA (Avery *et al.*, 1944) and, since then, the term 'transformation' acquired a genetic context. The subsequent discovery of bacteriophages (reviewed in Duckworth, 1976) and plasmids (Lederberg, 1952) has laid the foundation for the development of contemporary molecular biology, which has contributed enormously to many fields of biology, medicine, and biotechnology. The understanding of the fundamentals of the structure-and-function of mobile genetic elements as well as the enzymes of DNA metabolism allowed humans to adapt these mechanisms to serve their own needs. In the form of genetic engineering technology, these mechanisms are actively employed in a number of applications in science, biotechnology, and everyday life.

In a parallel development, the discovery and application of antibiotics beginning from the early 1940s has revolutionized the branch of clinical microbiology aimed at control and treatment of infectious diseases. Indeed, antibiotics are one of the most successful forms of chemotherapy known in medicine. Infectious diseases, which were the leading causes

of human morbidity and mortality for most of the human existence, were placed under control, and many millions of lives were saved thanks to antibiotic interventions. Initially rare, antibiotic resistance among pathogens emerged as a major problem in the 1950s when Japanese hospitals encountered *Shigella* dysentery outbreaks that resisted to treatment by usual antibiotics. Intensive clinical and genetic investigations performed by the Japanese scientists during that period resulted in a concept of episome-mediated transfer of drug resistance in the Enterobacteriaceae (Watanabe and Fukasawa, 1961; Watanabe, 1963). Moreover, this research also resulted in the eventual use of antibiotic resistance markers for genetic manipulations and was, therefore, one of the cornerstones for the development of genetic engineering.

Clinical studies focused on molecular mechanisms of antibiotic resistance, genetic elements involved and epidemiology have been developing in parallel to, but not interactively with, the environmental studies of HGT. The first attempts to estimate the frequencies of HGT in natural settings were performed in the 1970s with the use of model bacteria, *E. coli* and *Bacillus subtilis* (Weinberg and Stotzky, 1972; Graham and Istock, 1978). Since then, the approach involving the microcosm-based and field studies as well as a range of MGEs has resulted in a better understanding of how the environmental factors contribute to HGT in natural ecosystems (Van Elsas *et al.*, 2000; Timms-Wilson *et al.*, 2001). The analysis of gene exchange processes in natural settings was facilitated by the introduction of a variety of molecular ecology tools into the microcosm and field studies. These were the markers that allowed distinguishing the donor, recipient and transformant/transconjugant/transductant populations; DNA hybridization and sequencing; PCR typing; and others (Akkermans *et al.*, 1995; Götz *et al.*, 1996; Smalla *et al.*, 2000; Timms-Wilson *et al.*, 2001). The visualization of gene transfer *in situ* was made possible with the use of the *gfp* gene (Christensen *et al.*, 1996; Dahlberg el., 1998a; Andersen *et al.*, 1998). These technological advances allowed estimating the actual rates of HGT and factors affecting these rates in natural ecosystems.

Further substantiation for the important role played by HGT in the evolutionary process, especially among the Bacteria, came during the last decade, which is commonly referred to as the 'omics' era. With the advent of high-throughput sequencing it becomes possible to determine the genomic structure of many living organisms and apply the retrospective approach to HGT studies. Comparative genomic analyses revealed that, besides the core genes encoding essential cellular functions, the substantial part of bacterial genomes consists of auxiliary genes acquired by horizontal gene transfer (Ochman *et al.*, 2000). The latter group of genes may confer adaptive advantages under certain growth or environmental conditions that may contain antimicrobials, xenobiotics, heavy metals, sucrose, and other compounds. These genes also confer the important characteristics allowing the colonization of new ecological niches governed by biotic factors such as the symbiotic and pathogenic relationships (Koonin *et al.*, 2001).

Conceptual and classification developments

The range of MGEs involved in the evolution and adaptation of bacteria through HGT is continuously updated and re-classified according to a better understanding of HGT mechanisms. In addition to the 'classical' MGEs such as plasmids, bacteriophages and transposons, the list now includes various genomic islands conferring pathogenic and symbiotic properties, large megaplasmids comparable in terms of size with bacterial chromosomes, and other

elements with less defined structure-and-function. Owing to the ever extending list of newly described MGEs, it has been proposed to unify the heterogeneous classes of MGEs, such as conjugative transposons, integrative 'plasmids', genomic islands and numerous unclassified elements into integrative and conjugative elements (ICEs) (Burrus *et al.*, 2002). The basis for this re-classification was that these elements share similar characteristics, such as the excision by site-specific recombination, transfer by conjugation and integration by recombination between a specific site of elements and a site in the host's genome. Thus, ICEs combine the features of other MGEs, such as bacteriophages (integration into and excision from the host chromosome but no transmission by conjugation), insertion sequences (IS) and simple transposons (integration into and excision from the chromosome but no horizontal transfer), and conjugative and mobilizable plasmids (transferred from cell to cell by conjugation but replicated autonomously) (Wozniak and Waldor, 2010). A recent, even more radical, approach is to bring a number of MGEs under the umbrella of genomic islands (GEIs) (Juhas *et al.*, 2009). According to this view, GEI would encompass other categories of elements, such as ICEs, integrated plasmids, non-replicative but excisable elements (non-replicating *Bacteroides* unit, NBU; Shoemaker *et al.*, 2000), and even cryptic or damaged prophages.

Another interesting class of MGEs is the so-called gene transfer agent (GTA), which was initially described in *Rhodobacter capsulatus* (formerly *Rhodopseudomonas capsulata*) (Yen *et al.*, 1979) but later was found in many other bacteria (Lang and Beatty, 2007; Stanton, 2007). Although GTAs are similar to bacteriophages, they differ from them in two important aspects: (i) CTAs function only in genomic DNA transfer between cells; and (ii) there are no negative effects associated with gene transfer to the recipient. The representatives of another class of MGEs, named IS*CR*s, are known to move by a process called 'rolling-circle replication', and a function of this process is the concomitant movement of adjacent DNA upstream of their transposase genes (Toleman *et al.*, 2006). A recently discovered novel mechanism of HGT in *E. coli*, which was named 'cell-to-cell transformation', involves cell-derived DNA and, similar to Gram-positive bacteria, a putative promoting pheromone (Etchuuya *et al.*, 2011). These examples suggest that there is a continuum of MGEs, partially overlapping in their structure-and-function, rather than separate and strictly defined MGE classes.

Even the concepts within the well-established areas of MGE research such as the plasmid biology may require further clarifications and adjustments in the light of novel information coming from a broader sampling of microbial diversity and the corresponding genetic mechanisms associated with this diversity. For example, the original classification of plasmids into compatibility groups was based on the ability of plasmids to stably coexist in the same bacterium (Datta and Hedges, 1971). Molecular mechanisms underlying the incompatibility were explained essentially as a stochastic phenomenon based on the inability of a bacterial host to differentiate among similar replicons and thus giving rise to homoplasmid segregants as a direct consequence of random assortment during cell division (Novick, 1987). Within the frames of this concept, it is hard to explain the stable inheritance of multiple large plasmids in the Alphaproteobacteria (Pradella *et al.*, 2010). The implementation of a phylogenomic approach to plasmid classification suggests that the Rhodobacterales harbour a set of at least 18 compatible plasmids, which can in principle stably coexist within the same cell (Petersen *et al.*, 2009, 2011). Another concept that is presently undergoing an extensive reassessment is the role of prophages in bacterial genomes. The conventional

view of prophages as time bombs that are ready to enter the lytic cycle and destroy the host is gradually shifting towards their perception as a key component in bacterial survival strategies (Paul, 2008). Indeed, the prophages may confer many beneficial properties to the host helping to withstand osmotic, oxidative and acid stresses, as well as contributing to increased growth and biofilm formation (Wang *et al.*, 2010). In mammals, herpesvirus latency confers a symbiotic protection against the infection with the bacterial pathogens *Listeria monocytogenes* and *Yersinia pestis* (Barton *et al.*, 2007).

The recent record of rapid MGE-mediated dissemination of antibiotic resistance genes among bacterial pathogens also gave a substantial boost to revisit the conceptual framework of antibiotics and antibiotic resistance. Historically, this research was an enclave within the boundaries of clinical microbiology and bacterial genetics because it was thought that the antibiotic resistance problem was due to, and could be resolved within, the antibiotic treatment practices of human infectious diseases. Also, a contributing factor to the isolation was the little interaction with other fields of microbiology. The realization of the point that antibiotic resistance research should take a broader than the clinical microbiology approach to identify the factors ultimately leading to the acquisition of antibiotic resistance by pathogens entered the mainstream thinking substantially later. In particular, the placement of the problem within the evolutionary and ecological contexts appeared to be especially fruitful (Davies *et al.*, 2006; Aminov and Mackie, 2007; Fajardo and Martínez, 2008; Aminov, 2009, 2010). Two main arguments emerged from this synthesis: firstly, it is the enormous diversity of antibiotic resistance genes existing in the environmental microbiota that has accumulated during billions of years of evolution; and, secondly, the realization that there are no barriers among the ecological compartments in the microbial world, and that the microbiota of different compartments may easily exchange the gene pool through the MGE-mediated HGT.

Potential obstacles in HGT research

While exploring the wealth of data collected by environmental genomics/metagenomics we may get a glimpse of the HGT events of the past, we also understandably wish to know the extent of HGT in the present-day ecosystems, especially in the face of pressing needs such as the acquisition of antibiotic resistance by pathogens or the potential risks associated with genetically modified organisms (GMOs). Many microcosm and field experiments that are aimed at the estimation of HGT rates in natural ecosystems rely on model organisms with well-developed genetics. These organisms, however, have been selected as models owing to their features that are important for working under laboratory conditions, such as the simplicity of cultivation, tolerance to high nutrient concentration, the ease of genetic manipulation, and other characteristics that are not necessarily significant for the survival and multiplication in natural ecosystems. Laboratory strains of *Bacillus subtilis,* for example, have been selected and manipulated to become highly competent, which is important for bacterial genetics studies (Anagnostopoulos and Spizizen, 1961). Many wild-type strains of *B. subtilis,* however, are not naturally competent, and the transformation of these 'undomesticated' strains is possible only by highly unnatural means such as protoplast electroporation (Romero *et al.*, 2006).

Another problem which may be encountered when accessing HGT in natural ecosystems is that different MGEs may potentially interact with each other, and thus it is important to know the diversity of indigenous MGEs in microcosm and field studies. And finally, owing

to dispersion and population sizes, microorganisms can be transferred across distant environments (Hooper *et al.*, 2008). This geographical component may substantially contribute to HGT, especially if the genetic material transferred confers the traits important for the survival and reproduction in the 'donor' ecological niche. Thus, a certain degree of caution must be exercised when estimating the frequencies of HGT in natural ecosystems.

In the following two parts of this chapter, HGT is viewed mostly from an ecological and environmental regulation prospective rather than focusing on genetics and regulation at the cellular level. In the first part, the soil, aquatic, gut and biofilm communities are discussed in respect to HGT processes in these ecosystems. In the second part, factors potentially affecting HGT in natural ecosystems are analysed.

HGT in natural ecosystems

Soil microbiota

Soil ecosystems harbour an extremely broad diversity of microbiota reflecting plant type, soil type, soil management regime, and a number of other biotic and abiotic factors (Garbeva *et al.*, 2004). To begin with, the physical environment of soil is very heterogeneous in terms of gaseous, liquid or solid phases (Smiles, 1988). Additional variables include abiotic factors such as temperature, pH, concentration of nutrients and oxygen and moisture content and biotic factors such as antagonistic, commensal, mutualistic and other relationships among the soil inhabitants. Since the regulation of genetic machinery is highly responsive to the environment, these factors may indeed affect the frequencies of HGT. For example, the rate of conjugal plasmid transfer in soil varies depending on abiotic factors, such as soil moisture and temperature (Richaume *et al.*, 1989), pH (Rochelle *et al.*, 1989), and soil type (Richaume *et al.*, 1992). Water movement in soil may influence the survival and transport of genetically engineered strains (Trevors *et al.*, 1990). As for biotic factors, the presence of earthworms, protozoa and fungi certainly affects the conjugal plasmid transfer in soil (Daane *et al.*, 1996, 1997; Sørensen *et al.*, 1999, 2000). In general, bulk soil is generally poor in nutrients, and the active metabolic state of bacteria required for HGT is possible in nutritional hot spots such as the rhizosphere, the phyllosphere, decaying plant and animal tissue, and in manure-applied soil (Van Elsas and Bailey, 2002).

Indeed, conjugative plasmid transfers among plant phytosphere inhabitants have been detected on multiple occasions (Van Elsas *et al.*, 1988; Lilley *et al.*, 1994; Björklöf *et al.*, 1995; Pukall *et al.*, 1996; Lilley and Bailey, 1997; Kroer *et al.*, 1998). The most important factors contributing to the frequency of horizontal plasmid transfer in the rhizosphere appeared to be root growth and exudate production (Mølbak *et al.*, 2007). Transfer ratios were about 10 times lower in control soil than in the pea and barley rhizospheres. For some plasmids, an exceptionally broad range of recipients in the rhizosphere was demonstrated (Musovic *et al.*, 2006). A high-frequency conjugal transfer of an IncP-1 plasmid pKJK10 was shown not only to bacteria belonging to the alpha, beta, and gamma subclasses of the Proteobacteria, but also to *Arthrobacter* sp., a Gram-positive member of the Actinobacteria. Compared with filter mating, the plant model (alfalfa sprouts) provided an environment substantially enhancing the transfer of plasmid- and transposon-encoded antibiotic resistance markers between lactic acid bacteria (Toomey *et al.*, 2009).

Application of manure to soil is another hot spot contributing to the increase in local

concentrations of MGEs and the frequency of HGT in this ecosystem. This practice, as it has been shown, may enhance plasmid mobilization and survival of the introduced donor strain (Götz and Smalla, 1997). If applied together with an antibiotic, the effect is synergistic and affects the transfer frequencies and composition of MGEs introduced with manure (Heuer and Smalla, 2007). Field application of piggery manure, which harbours a substantial reservoir of broad-host range plasmids conferring multiple antibiotic resistance genes, results in the dissemination of IncN, IncW, IncP-1, and pHHV216-like plasmids into agricultural soils (Binh *et al.*, 2008). Clinically relevant class 1 integrons are also introduced into soil via similar practices (Binh *et al.*, 2009).

Besides contributing to the accumulation of MGEs and to the HGT increase, the application of manure to soil has other important consequences such as the generation of novel MGE diversity and dissemination of novel phenotypes (for example, resistance to recently introduced antibiotics) among bacterial populations. An example of this kind was described recently (Heuer *et al.*, 2009) and deserves a more detailed discussion. In this work, a novel plasmid type with 36% G+C content was captured from manure-treated soil microbiota by conjugation to *E. coli* recipient. The core of plasmids of this type is probably the product of recombination, comprising transfer and maintenance genes with moderate homology to plasmid pIPO2 and a replication module (rep and oriV) of other descent, correspondingly. These plasmids encode a number of antibiotic resistance genes including *tet*(X) (GenBank accession number FJ012881), which may confer resistance against a recently introduced third-generation tetracycline, tigecycline (Moore *et al.*, 2005). The species of *Acinetobacter* are the putative hosts for these low G+C plasmids in soil ecosystem (Heuer *et al.*, 2009), which is of concern because the closely related multidrug-resistant *Acinetobacter baumannii* is one of the currently emerging threats in hospitals (Dijkshoorn *et al.*, 2007). Tigecycline is currently proposed as a new treatment choice against *A. baumannii* (Bosó-Ribelles *et al.*, 2008) but this bacterium already poses a significant problem with the easily emerging resistance during tigecycline therapy, albeit conferred by a mechanism other than TetX (Peleg *et al.*, 2007; Damier-Piolle *et al.*, 2008). The similarity of structural and replication features of low G+C plasmids from soil to the corresponding plasmids of clinical *A. baumannii* isolates, together with the stable inheritance of these plasmids in *Acinetobacter* spp. (Heuer *et al.*, 2009), suggests that, once acquired, the plasmids may be easily accommodated by *A. baumannii* in the clinical environment. Given the carriage of resistance against the drug of last resort by these plasmids, their possible entry into clinical *A. baumannii* is a highly undesirable scenario. Disappointingly, there is some evidence that at least some clinically relevant resistance genes have an environmental origin (Wright, 2010) suggesting past horizontal gene exchange events between these ecological compartments. Thus, the microbiota of soil, especially of manure-fertilized soil, harbours a wide variety of MGEs enabling extensive HGT within and among microbial ecosystems.

At the same time, nutritionally poor soil ecosystems may also be involved in the HGT process. For example, a deep terrestrial subsurface, which is highly oligotrophic and extreme in terms of physical and chemical conditions, still harbours bacterial populations that carry MGEs and the genetic signatures of past gene transfer events, some of them apparently recent (Coombs and Barkay, 2004; Coombs, 2009). It is still more likely that the HGT rates under the nutritionally favourable conditions are higher owing to the substantial biosynthetic and energetic requirements for conjugation, DNA uptake, and lytic cycle.

Recent assessments of several types of soil revealed that lysogeny is relatively common

in soil microbiota (Williamson *et al.*, 2007; Ghosh *et al.*, 2008; Williamson *et al.*, 2008). Similar to the plasmid-mediated HGT, the diversity and dynamic of bacteriophages is also mostly confined to the nutritionally rich hot spots such as the rhizosphere. The decline in the introduced fluorescent *Pseudomonas* sp. population revealed the presence of large numbers of bacteriophage in the sugar beet rhizosphere (Stephens *et al.*, 1987). On the other hand, the growth and interaction dynamics of streptomycetes and a bacteriophage, which was investigated under a less nutritionally rich condition of soil microcosm, did not reveal such an effect of phages (Burroughs *et al.*, 2000). No measurable impact on the host in terms of reduced growth by the phage was found under these conditions. Interestingly, the burst size of the phage was larger in soil relative to that observed in liquid culture, suggesting that *in vitro* transduction experiments may underestimate the impact of this particular HGT mechanism in natural ecosystems.

In the soil ecosystem, bacteriophages display local adaptation to their bacterial hosts (Vos *et al.*, 2009). Sympatric phages are more infective than are phages from samples some distance away, suggesting that the phage-mediated HGT is highly localized in soil compartments. In model microcosm experiments, the presence of phages greatly reduced the sympatric diversity of the host bacterium but favoured the allopatric host diversification (Buckling and Rainey, 2002).

The possibility of natural genetic transformation of *B. subtilis* and *Pseudomonas stutzeri* has been demonstrated in a soil/sediment model system (Lorenz *et al.*, 1988; Lorenz and Wackernagel, 1990). These studies found significantly higher rates of transformation if the transforming DNA was associated with mineral/particulate material and thus protected against the nuclease degradation. The protective effect of DNA absorption on minerals comprising soil and sediments against DNases has been noticed on many occasions (Aardema *et al.*, 1983; Romanowski *et al.*, 1991; Demanèche *et al.*, 2001; Cai *et al.*, 2006). While some naturally competent bacteria, as in the examples above, indeed demonstrate a certain level of transformability, the occurrence of naturally transformable bacteria amongst bulk and rhizosphere soil microbiota is very low (Richter and Smalla, 2007). Various transformation assays were performed, but only transformants with a positive control, *Acinetobacter baylyi* BD413, which is a highly transformable strain and can be transformed by DNA from virtually any sources, were obtained. Thus, the authors concluded that the proportion of native rhizosphere and bulk soil bacteria, which are naturally transformable, is negligibly low.

There have been (and indeed there still are) intensive debates surrounding the issue of possible risks associated with the release of genetically modified organisms (GMOs) into the environment, in particular, transgenic plants. Studies on persistence of plant DNA in soil arrived at different conclusions depending on the experimental setup. In microcosm experiments modelling *in situ* transport of recombinant plant DNA (rDNA) from Roundup Ready (RR) maize and soybean by leachate water, half-lives of rDNA in leachate water ranged from 1.2 to 26.7 h, depending on the temperature (Gulden *et al.*, 2005). The presence of transgenic DNA in soil where RR maize and soybean were cultivated can be detected by real-time PCR for up to 1 year after seeding (Lerat *et al.*, 2007). In soil microcosm experiments, recombinant DNA entry into soil from the decomposing RR leaf biomass was investigated, and the corresponding DNA was detectable in soil after 30 days (Levy-Booth *et al.*, 2008). At the present stage of our knowledge, HGT from transgenic plants to terrestrial bacteria is considered to be a rare event (Nielsen *et al.*, 1998). A recent study with the use of an *in situ* visualization technology, nevertheless, demonstrated this process could be observed in real

time (Pontiroli *et al.*, 2009). This study, however, employed a highly transformable *A. baylyi* strain BD413, which may lead to the overestimation of natural transformation rates. The current view is that even if HGT from transgenic plants to soil microbiota takes place, it is not expected to influence prokaryotic evolution or have negative effects on human or animal health and the environment (Brigulla and Wackernagel, 2010).

Microbiota of aquatic ecosystems

More than 70% of the earth surface is covered with water, and the World Ocean is one of the principal components forming the climate and biosphere of the Earth. As the largest habit on the Earth, the World Ocean hosts a large diversity of life and is responsible for nearly the half of oxygen production. Besides, there are many freshwater ecosystems, such as lakes, rivers, and smaller water bodies.

One of the earliest accounts on the occurrence of antibiotic resistance in marine bacteria has found that this phenotype is common within this microbiota (Sizemore and Colwell, 1977). Interestingly, the resistance phenotype was more frequently encountered in bacteria from seawater samples collected offshore than for those collected near shore. Antibiotic resistance was even present in bacteria collected about 522 km offshore and at depths of 8200 m. In 6 out of 10 bacterial isolates the presence of plasmid DNA was confirmed by ethidium bromide-caesium chloride density gradient centrifugation. In general, however, antibiotic resistance phenotype and the presence of plasmids in marine bacteria tend to correlate with the degree of pollution, especially by toxic chemical waste (Baya *et al.*, 1986; Young, 1993). A recently applied cultivation-free approach for characterization of coastal *Synechococcus* metagenome relied on flow cytometry-based sorting of cells with a subsequent 454-shotgun pyrosequencing (Palenik *et al.*, 2009). This approach allowed identifying novel plasmids that were not found in model strain genomes of this clade. Plasmids with an enormous size range are also widespread in the *Roseobacter* clade, the representatives of which constitute up to 25% of the total marine bacterial community and thus play a global role in this ecosystem (Brinkhoff *et al.*, 2008). Some representatives may carry up to 12 extrachromosomal replicons, suggesting a very sophisticated partition mechanism ensuring their stable inheritance (Pradella *et al.*, 2010). In marine *Vibrio* the majority of plasmids are associated with pathogenic or symbiotic relationship with the host organism (Hazen *et al.*, 2010).

With the advent of high-throughput sequencing technologies, it becomes possible to characterize the marine phage diversity without the isolation and cultivation steps, which are extremely time- and resource-consuming while the outcome is limited to a few phages that we are able to multiply and characterize under laboratory conditions. In fact, the metagenomic approach is the only way to characterize the viral diversity because there is no single gene common to all phages to serve as a phylogenetic marker (Edwards and Rohwer, 2005). Comparative marine virome analyses involving four oceanic regions demonstrated that the global diversity is very high – presumably several hundred thousands of species (Angly *et al.*, 2006). The oceanic regions had different assemblages of marine phages suggesting strong local selective pressures enriching for certain viral types. Metagenomic characterization of viruses within aquatic microbial samples revealed a prevalence of genes encoding microbial physiological functions among viral sequences (Williamson *et al.*, 2008). Screening of 113 marine bacterial genomes for prophages yielded 64 prophage-like elements, 21 of which strongly resembled GTAs (Paul, 2008). Hence, the viral- and GTA-mediated HGT

is a common mechanism for generating microbial diversity in the marine environment thus contributing to the survival in different parts of this extensive ecological niche. Moreover, marine phages may directly contribute to the fitness of their hosts. It has been suggested, for example, that the auxiliary genes in marine phages may confer selective advantage to the host through the viral gene cassettes encoding core photosystems, I and II (Sharon *et al.*, 2009). In a more recent work from the same group the repertoire of host genes detected in marine viruses appeared to be even broader including genes of energy metabolism as well as involved in translation and post-translational modification (Sharon *et al.*, 2011).

Historically, the *in situ* HGT rates in aquatic ecosystems have been typically studied with the use of microcosms, and it is understandable that in a number of cases these models have certain limitations in reproducing the vast range of ecological niches present, for example, in the World Ocean. In most cases, the microcosms are limited to modelling the shallow costal or estuarine ecosystems, frequently without the presence of ambient macro- and microbiota. Laboratory experiments also lack the scale of natural ecosystems and, as a consequence, may discount the role of important variables potentially affecting HGT. Another aspect, which needs to be taken into consideration, is the use of model donors and recipients that have been 'preselected' under laboratory conditions to monitor HGT. The HGT results obtained with the use of model organisms, genetic constructs and microcosms should be interpreted with a reasonable degree of precaution when extrapolating them in an attempt to describe a broader environment.

It has been shown that the presence of marine sediments in microcosms facilitates the uptake and expression of exogenous DNA by transformable marine *Vibrio* sp. (Stewart and Sinigalliano, 1990). It was concluded from this study that sediments were more likely niches for natural transformation than the water column in the marine environment. However, another study of natural plasmid transformation of a *Vibrio* strain in marine water column and sediment microcosms, in the presence of the ambient microbial community, arrived at the opposite conclusion (Paul *et al.*, 1991). The authors explained this discrepancy by a differential experimental setup involving the presence of ambient microbiota in their experiments, while the previous work used sterile sediments. Estimation of transformation rates in estuarine environments based upon the distribution of competency and transformation frequencies in isolates and mixed populations ranged from 5×10^{-4} to 1.5 transformants per day (Frischer *et al.*, 1994). Another aspect of natural transformation is that the structure-and-function of transforming DNA may be affected by the *in situ* microbiota. For instance, a broad-host-range plasmid pQSR50 that was introduced into the indigenous marine bacteria by natural transformation was subjected to the alteration of restriction profiles (Williams *et al.*, 1997). This involved changes in methylation patterns as well as structural rearrangements of the plasmid following gene transfer, thus contributing to the generation of plasmid diversity among the *in situ* bacterial populations.

Conjugative plasmid transfers under simulated marine environment conditions have been demonstrated in many experiments. Transconjugants can be detected even under oligotrophic conditions and at very low population densities of donors and recipients (Goodman *et al.*, 1993). The heterogeneity of the marine environment affects the HGT rates; the plasmid transfer frequency is higher among cells attached to the bead surfaces in the biofilm than among cells in the aqueous phase (Angles *et al.*, 1993). The use of the *in situ* technology with the *gfp*-tagged conjugative plasmid pBF1 from *Pseudomonas putida* suggests that the directly determined rates of horizontal plasmid transfer in marine bacterial

communities may be high, ranging from 2.3×10^{-6} to 2.2×10^{-4} transconjugants per recipient (Dahlberg *et al.*, 1998b).

In one of the initial studies, transduction of *P. aeruginosa* streptomycin resistance by a generalized transducing phage, F116, was monitored in a flow-through chamber suspended in a freshwater reservoir (Morrison *et al.*, 1978). The frequency of F116-mediated transduction ranged from 1.4×10^{-5} to 8.3×10^{-2} transductants per recipient during the 10-day incubation period. A recent investigation of phage-mediated gene transfer in freshwater environments used a more advanced technological tool to monitor these events at the single-cell level; the so-called cycling primed *in situ* amplification-fluorescent *in situ* hybridization (CPRINS-FISH) (Kenzaka *et al.*, 2010). The P1, T4 and EC10 phages mediated gene transfer from *E. coli* to both plaque-forming and non-plaque-forming Enterobacteriaceae strains at frequencies of $0.3–8 \times 10^{-3}$ per plaque-forming unit (PFU). The rate of transfer of EC10 ranged from undetectable to 2×10^{-3} per total direct cell count when natural bacterial communities were the recipients. This suggests that transduction may involve a wide range of bacteria, not necessarily limited by close relatives, and that the phage-mediated HGT is a frequent event in freshwater environments.

Compared to the freshwater microcosm studies, the corresponding experiments imitating the marine environment yielded lower frequencies of plasmid transduction in the mixed bacterial communities; the values were in the range from 1.58×10^{-8} to 3.7×10^{-8} transductants/PFU (Jiang and Paul, 1998). This is not to say that the overall HGT rates in marine ecosystems are low. Other MGEs residing in marine microbiota may generate an extraordinarily extensive gene flow – one of the highest ever detected. A recent study demonstrated extremely high rates of HGT in marine ecosystems that are mediated by GTAs (McDaniel *et al.*, 2010). The frequencies detected are by many orders of magnitude higher than those of transformation or transduction, with as many as 47% of the culturable marine microbiota being confirmed as gene recipients. Given the wide presence of GTAs in phylogenetically and ecologically diverse bacteria (Stanton, 2007), GTA-mediated HGT may be more common in other natural ecosystems and may generate more extensive gene exchange than previously anticipated.

Comparative genomics and transcriptomics of marine bacteria are consistent with the high rates of HGT in these ecosystems. The co-occurring *Shewanella baltica* isolates from similar depths exchanged a larger fraction of their core and auxiliary genome compared with the isolates from more different depths (Caro-Quintero *et al.*, 2011). These HGT events took place in the very recent past reflecting the rapid adaptation to environmental settings through gene acquisition. Genomic evolution for a cold marine lifestyle and *in situ* explosive biodegradation in *Shewanella* spp. also involved an extensive gene acquisition from deep-sea bacteria (Zhao *et al.*, 2010). A recent study of global population structure of *Vibrio cholerae*, which is a widely distributed microbial species in the coastal ocean regions, revealed a rapid diversification among geographically distant populations due to the extensive local gene exchange (Boucher *et al.*, 2011). This integron-mediated HGT involves mostly the gene pool encoding secondary metabolism and cell surface modification thus allowing rapid adaptation to local ecological niches.

Most of our knowledge about the HGT events in aquatic ecosystems comes from studies of the resident bacteria and their corresponding MGEs. Much less is known, however, about the corresponding mechanisms and events taking place in the other procaryotic domain of life, Archaea. The explanations for this may be due to a much smaller absolute number

of representatives from this taxonomic group as well as their adaptation to the extreme environments making it difficult to study them under laboratory conditions. Nevertheless, the advent of cultivation-free approaches such as metagenomic analysis allowed uncovering the past HGT events engraved in the archaeal genomes. Detailed phylogenetic analysis of the 41 genes present in a genome fragment of a marine planktonic group I archaeon, DeepAnt-EC39, revealed that 11 of them had been most likely acquired by HGT from different sources, including bacteria and other unrelated archaea (López-García *et al.*, 2004). The possession of a proteorhodopsin gene by marine planktonic archaea is also likely owing to the past acquisition from bacteria (Frigaard *et al.*, 2006). Metagenomic analysis of marine uncultured planktonic archaea revealed frequent HGT acquisition, mostly from bacterial donors but also from other archaea and eukaryotes (Brochier-Armanet *et al.*, 2011). Interestingly, the laterally transferred gene pool is conserved within the several representatives of the studied groups suggesting the important role played by HGT in the adaptation of these archaea to the cold and nutrient-depleted deep marine environment. Genomic plasticity of the psychrophilic archaeon, *Methanococcoides burtonii*, driven, in particular, by HGT and transposase activity is also thought enabled its adaptation to the cold Antarctic lake environment (Allen *et al.*, 2009). On the other side of the temperature extreme, the transposition, genome rearrangements and HGT events are also seen as a necessary adaptation of *Pyrococcus* species to the shallow hydrothermal vent ecosystem (Hamilton-Brehm *et al.*, 2005; White *et al.*, 2008). In general, archaeal populations of aquatic ecosystems demonstrate the same tendency as bacteria to form the local mobilomes and gene pools, allowing a better adaptation to a particular ecological niche.

Intestinal microbiota

The frequencies of horizontal gene transfer by conjugation are usually estimated using standard mating techniques, in liquid culture or on the surface of solidified media or filters. The microcosm and field experiments that are the better estimates of HGT in the environment usually deal with lower population densities, lower nutrient availability, and generally lower temperatures. The *in vivo* conditions, however, are substantially different, especially in the intestine, where the enormously dense and diverse microbiota performs a number of functions important for the host organism. These functions include the prevention of colonization by pathogens, degradation of dietary (polysaccharides) and *in situ*-produced (mucin) compounds, production of nutrients (short chain fatty acids and vitamins), shaping and maintenance of normal mucosal immunity, and contribution to intestinal epithelial homeostasis. The commensal microbiota is under constant surveillance by the innate (antimicrobial peptides) and adaptive (immunoglobulins) immunity. The effect of innate immunity breaches on gut microbiota can be seen in genetically susceptible hosts, the commensal microbiota of which is formidably restructured compared to normal subjects (Khachatryan *et al.*, 2008).

The discovery of ICEs among the representatives of the Bacteroidetes, the major bacterial phylum in the mammalian gut, brought the notion that gene transfer in this ecosystem may be intense (Salyers, 1993), and gut microbiota, therefore, may represent one of the major reservoirs for antibiotic resistance genes (Salyers *et al.*, 2004). Indeed, the taxonomically different representatives of gut microbiota may share the pool of closely related antimicrobial resistance genes (Frye *et al.*, 2011). The role of bacteriophages in acquisition of genes by pathogenic gut microbiota and subsequent evolution towards pathogenicity

also pointed to HGT as a crucial event in the development of virulence traits and antibiotic resistance (Calderwood *et al.*, 1987; Barondess and Beckwith, 1990; Brabban *et al.*, 2005). More recent metagenomic studies of the human gut microbiota allowed estimating the role of MGEs without the cultivation bias. The uncultured viral community from human faeces contained an estimated 1200 viral genotypes (Breitbart *et al.*, 2003). Interestingly, unlike the similarity of gut bacterial communities among genetically related individuals, the viral communities are unique to individuals regardless of their degree of genetic relatedness (Reyes *et al.*, 2010). Besides viromes, a recent metagenomic inventory identified a conjugative transposon family explosively amplified in human gut microbiomes (Kurokawa *et al.*, 2007). The culture-independent TRACA system was used to sample plasmid diversity in the human gut microbiota (Jones *et al.*, 2010). This study suggested a broad global distribution of some plasmids and plasmid families that are potentially unique to the human gut microbiome. Thus these findings further supported the earlier cultivation-based views that the intestinal microbial ecosystem is extremely enriched by MGEs thus making it the arena of a potentially extensive gene exchange. Indeed, the range of genes that have been exchanged in the past is not limited to the virulence and antibiotic resistance genes but also includes the genes of the core gut microbiome such as encoding bile salt hydrolases (Elkins *et al.*, 2001) or butyrate metabolism enzymes (Louis *et al*, 2007).

Humans and agricultural animals are the main consumers of antibiotics, for therapeutic, prophylactic and growth-promoting purposes and, as it will be discussed in the next part, antibiotics may substantially increase the rates of HGT. But what could be other host-mediated factors contributing to the maintenance and transfer of MGEs in gut microbiota? It seems that the *in vivo* environment itself may enhance the transfer frequency and contribute to the stable inheritance of MGEs even in the absence of selection by antibiotics (Johnsen *et al.*, 2002; Dahl *et al.*, 2007). Thus, *in vitro* models may substantially underestimate the transfer potential of MGEs. In nutritionally poor environments, such as bulk soil, the presence of earthworms greatly enhances the transfer of plasmid pJP4 from an inoculated donor bacterium, *P. fluorescens*, to the indigenous soil microbiota (Daane *et al.*, 1996). Other soil microcosm experiments, modelled with *E. coli* as a donor of a genetically marked large conjugative plasmid RP4luc, in the presence or absence of earthworms, provided evidence that the gut passage was a precondition for a plasmid transfer to soil microbiota (Thimm *et al.*, 2001). Interestingly, the plasmid was transferred at higher frequencies than detected in filter mating, suggesting once again that the HGT rates in nature may be higher than the laboratory estimates. This observation also confirms the earlier notion that microbial ecosystems are not isolated and there is a potential for lateral gene exchange among different microbial ecosystems. If MGEs from soil have entered the earthworm gut, then they can also enter the gut of animals that are next in the food chain, for example, moles and birds.

Another curious factor that may contribute to the enhanced HGT is the presence of ciliates. Ciliates are common in many aquatic ecosystems as well as in the gastrointestinal systems such as the rumen. Their food vacuoles are formed through phagocytosis and follow a particular path through the cell resembling a primitive gastrointestinal tract. It has been shown that ciliates may enhance the rate of conjugal transfer between *E. coli* strains by two orders of magnitude, and the mechanism involved is the accumulation of bacteria in vesicles (Matsuo *et al.*, 2010). The mechanism described may contribute to the dissemination of antibiotic resistance in bacterial populations (Oguri *et al.*, 2011).

The insect gut can also be considered as a hot spot contributing to the enhanced HGT

in this ecosystem. For example, the rates of conjugative plasmid transfer between *S. enterica* Newport and *E. coli* in the gut of the lesser mealworm beetle are by two orders of magnitude higher compared to filter mating (Poole and Crippen, 2009). The occurrence of conjugal plasmid transfer and transduction was also observed in the flea and house fly gut (Hinnebusch *et al.*, 2002; Petridis *et al.*, 2006; Akhtar *et al.*, 2009). An assessment of natural transformability of bacteria in the insect gut, however, failed to detect any transformation event, even with the use of *A. baylyi* strain BD413 as a recipient (Ray *et al.*, 2007), thus suggesting that the natural transformation plays a minor role in the HGT in the gut of insects and, possibly, in the gut of other animals.

Conditions in the gut can be considered as very favourable for HGT. Firstly, the host provides a continuous inflow of nutrients that allow maintaining the active metabolism of gut microbiota. Secondly, the population densities are extremely high and thus conducive to the HGT mechanisms requiring intimate cell-to-cell contact such as conjugation. Thirdly, in a part of animals, with homeothermic metabolism, the constant body temperature may allows the bacterial cells to perform at optimal efficiency. And finally, the vast diversity of gut microbiota itself may have an 'amplification effect' for HGT (Dionisio *et al.*, 2002). In addition to these well-known factors, recent investigations have uncovered the mechanisms of host-microbe molecular crosstalk that may contribute to the frequencies of HGT in the gut (Mulder *et al.*, 2011).

One of these mechanisms is based on the ability of bacteria to sense and respond to host signals. In particular, bacterial sensing and responding to the level of host stress hormones is a well-established fact (Sperandio *et al.*, 2003; Clarke *et al.*, 2006; Karavolos *et al.*, 2008; Spencer *et al.*, 2010). Bacterial responses to the host stress may also involve a genetic component, which is expressed through the enhanced conjugative gene transfer between enteric bacteria (Peterson *et al.*, 2011). In these *in vitro* experiments, the physiological concentrations of noradrenaline stimulated the transfer of a conjugative plasmid from a clinical strain of *Salmonella* sp. to an *E. coli* recipient. Interestingly, the adrenergic receptor antagonists negated the stimulatory effect of noradrenaline on conjugation. These mediators of host stress may possibly affect HGT under the *in vivo* environment as well.

The issues of potential risks associated with the consumption of GMOs by humans and animals have been addressed in a number of feeding trials. In general, there is a lack of evidence that DNA of transgenic plants, in particular the markers used for their construction, can be taken up by gut microbiota or enter the organs other than the gastrointestinal tract. Neither small fragments of transgenic DNA nor immunoreactive fragments of transgenic protein were detectable in loin muscle samples from pigs fed a diet containing Roundup Ready soybean meal (Jennings *et al.*, 2003). An assessment of the survival of transgenic plant DNA in the human gastrointestinal tract concluded that gene transfer did not occur during the feeding experiment involving GM soya (Netherwood *et al.*, 2004). No traces of the construct or endogenous soybean DNA could be detected in muscle samples of rats fed soybean meal from Roundup Ready or conventional soybeans (Zhu *et al.*, 2004). Likewise, no traces of transgenic DNA were detected in the milk of cows fed maize silage from an herbicide-tolerant genetically modified variety (Phipps *et al.*, 2005). Plasmid and genomic DNA from GM plants were used in *in vitro* and *in vivo* (mono-associated rats) transformation studies, but no detectable transfer of DNA was found (Wilcks and Jacobsen, 2010). Attempts to detect DNA transfer from transgenic plants to bacteria in the intestine of the tobacco hornworm (Deni *et al.*, 2005) or bees were also unsuccessful (Mohr and Tebbe, 2007).

Microbiota of biofilms

Biofilms are the matrix-enclosed aggregates of microbiota, attached to each other and to biological or non-biological surfaces (Hall-Stoodley *et al.*, 2004). This ancient form of adaptation appeared very early in the prokaryotic phase of evolution being selected as a trait helping to withstand the hostile environmental factors. Indeed, biofilms can be perceived as a form of primitive multicellular organisms, which use the same strategy in their interaction with the environment as their eukaryotic counterparts do. This successful trait, therefore, was replicated in many bacterial and archaeal lineages; and biofilm communities are widespread in many natural ecosystems. It is now widely acknowledged that the majority of microbiota found in natural, clinical, and industrial settings persist in association with surfaces rather than in the planktonic state (Costerton, 1995; Davey and O'Toole, 2000). In natural ecosystems, biofilms are usually found in many aquatic or semi-aquatic ecosystems such as rocks and pebbles of most streams and rivers, on the surface of still water bodies, in wastewater treatment systems, in water and sewage pipes, in hot springs, in the subtidal and intertidal solid surfaces of marine ecosystems, on the teeth of humans and animals, in chronic infections of human body, and in many other ecosystems. For studies of HGT in highly organized structures as biofilms, technologies such as single-cell detection of donor, recipient and transconjugant bacteria, combined with individual-based mathematical models, have been developed to estimate the HGT rates *in situ* (Sørensen *et al.*, 2005). Raman spectrometry and environmental scanning electron microscopy analyses, combined with molecular ecology tools, allow a better understanding of structure-and-function of biofilms including the chemical composition of the matrix, microbiota embedded in the matrix, and the spatial distribution of biofilms (Schwartz *et al.*, 2009).

Most of our knowledge in regards to the biology of biofilms came from clinical microbiology. This increased attention to the clinical aspects of biofilm communities was primarily dictated by their role in human disease (Parsek and Singh, 2003; Fux *et al.*, 2005; Lindsey and von Holy, 2006; Estrela *et al.*, 2009; Kaplan, 2010). Biofilm formation is linked to the pathology of many infectious diseases, and biofilms are notoriously difficult to eradicate because of the increased level of resistance to antibiotics (Stewart and Costerton, 2001; Anderson and O'Toole, 2008; Høiby *et al.*, 2010a). The ability to form highly resilient biofilms has been demonstrated for many human pathogens such as *Pseudomonas aeruginosa* (for recent reviews, see Harmsen *et al.*, 2010; Hassett *et al.*, 2010; Häussler, 2010; Høiby *et al.*, 2010b), *Staphylococcus aureus* (Goerke and Wolz, 2010), *S. epidermidis* (Fey and Olson, 2010; Rohde *et al.*, 2010), *Streptococcus pneumoniae* (Moscoso *et al.*, 2009), *S. mutans* (Senadheera and Cvitkovitch, 2008), *Neisseria gonorrhoeae* (Greiner *et al.*, 2005; Falsetta *et al.*, 2009; Steichen *et al.*, 2011), *Campylobacter jejuni* (Haddock *et al.*, 2010; Naito *et al.*, 2010), *Candida* spp. (Morales and Hogan, 2010; Williams *et al.*, 2011), and others. Besides, microorganisms may form mixed-species communities and, in a number of cases, this may promote the development of biofilms (Bamford *et al.*, 2009; Silverman *et al.*, 2010; Teh *et al.*, 2010).

Given their structure-and-function, biofilms are the hot spots for HGT because they provide high population densities and close proximity of cells, cells in biofilms are metabolically active, and the microbiota is protected against harsh environment, predators and immune surveillance by the extracellular matrix in which the cells are encapsulated. Indeed, HGT occurs with enhanced efficiency in biofilms, and conjugative plasmids themselves contribute to the development, stabilization and expansion of biofilms (Hausner and

Wuertz, 1999; Ghigo, 2001; Molin and Tolker-Nielsen, 2003; Reisner *et al.*, 2006; Burmølle *et al.*, 2008). Conjugal plasmid transfer that is implemented through the synthesis of pili and type IV secretion system contributes to the intimate cell-to-cell contact, thus facilitating the formation and growth of biofilms. Type IV secretion systems use a pilus-based system to mediate DNA or protein transfer (Hayes *et al.*, 2010). The involvement of type IV secretion system in biofilm formation has been demonstrated for a number of bacteria (Shime-Hattori *et al.*, 2006; Li *et al.*, 2007; Luke *et al.*, 2007; Barken *et al.*, 2008; Varga *et al.*, 2008; Bahar *et al.*, 2009; Gibiansky *et al.*, 2010). Protection under the umbrella of biofilms confers a selective advantage for the bacteria as well as aids further plasmid/ICE/GEI transfers. Thus, this positive feedback sustains the diversity of MGEs in biofilms and offsets the fitness cost associated with the carriage of MGEs.

The presence of prophages in the host's genome may have a modulatory effect on biofilm formation and physiology of the host, including central metabolism (Wang *et al.*, 2009). Some prophage-encoded proteins are actually essential for biofilm formation (Toba *et al.*, 2011). The modulatory effect of prophages is expressed in the enhancement of biofilm formation and the resistance of biofilms against adverse environmental conditions (Carrolo *et al.*, 2010; Wang *et al.*, 2010). The biofilm matrix is a conglomerate of polymers usually consisting of extracellular DNA (eDNA), proteins, and polysaccharides. In this regard, a partial phage-mediated lysis of a proportion of microbiota in biofilm, due to spontaneous phage induction, may provide the eDNA supply, thus contributing to biofilm formation and maintenance by the remaining bacterial populations (Carrolo *et al.*, 2010; Gödeke *et al.*, 2011). At the same time, transmission electron microscopy reveals also the presence of intact bacteriophage particles that are enmeshed in the extracellular polymeric matrix of biofilms (Kay *et al.*, 2011). Precise deletion of *E. coli* prophages uncovered the role of e14 and rac proteins in the increased early biofilm formation (Wang *et al.*, 2010). Besides, other prophages contributed to the host resistance against a variety of stresses, including osmotic, oxidative and acid. These examples reveal the role of the phage-mediated HGT in bacterial evolution and adaptation, especially in regards to the acquired ability to form biofilms as well as to withstand environmental stresses.

Natural competence and transformability in bacteria usually correlates with the presence of type IV pili or type IV pilin-like proteins (Averhoff and Friedrich, 2003). While the role of conjugation machinery and pili in biofilm formation and subsequent acceleration of conjugative element transfer is firmly established, it is not clear if the same is true for natural transformation. Given the involvement of type IV pili or type IV pilin-like proteins in natural transformation and in biofilm formation, would this result in a higher rate of transformation within biofilms? Do the large quantities of eDNA in the extracellular matrix participate in natural transformation of bacteria in biofilms? There are very few works that have addressed these questions. Horizontal transfer of non-conjugative plasmids in *E. coli* colony biofilms suggested the possibility of natural transformation within bacterial biofilms (Maeda *et al.*, 2006). Later, however, it became clear that the mechanism of DNA acquisition in the biofilm is more complex and was, therefore, described as a 'cell-to-cell transformation', which also involves a peptide pheromone as a regulator (Etchuuya *et al.*, 2011). Other circumstantial evidence of DNA transfer due to close cell-to-cell contact (Van Randen and Venema, 1984; Wang *et al*, 2007) may be interpreted within the frames of this hypothesis, although the transfer in these cases was between the genera, and the role of pheromones was not shown. The language of intercellular communication used in regulation of various processes within

the complex structure of biofilms is indeed the language of pheromones such as N-acyl-L-homoserine lactones, furanosyl borate diester and peptide autoinducers (Dickschat, 2010). Recently, the possibility of transfer of tetracycline resistance by transformation with eDNA within model oral biofilms was described, although without specifying the frequency of such an event (Hannan *et al.*, 2010).

Factors influencing HGT

Stress and SOS response

It has been long recognized that various stress conditions may contribute to increased rates of HGT. For example, UV irradiation or starvation affects the mobility of transposons and insertion sequences (Levy *et al.*, 1993; Ilves *et al.*, 2001). While the lysogenic cycle in the phage development limits its inheritance to the vertical transfer within the chromosome of the host, the stress factors such as induction of the SOS response or amino acid deprivation of the host lead to the lytic cycle and further horizontal dissemination of phage and transduced DNA (Melechen and Go, 1980; Little, 2005). Control of horizontal transfer of ICEs, which are found in many bacterial genomes and which encode a variety of properties besides antibiotic resistance and virulence, may also be regulated through stress, SOS and other environmental signals (Auchtung *et al.*, 2005; Bose *et al.*, 2008). In particular, the SOS response, which is induced by DNA damaging agents such as mitomycin C and antibiotics such as fluoroquinolones and dihydrofolate reductase inhibitors, leads to the expression of the SXT activators resulting in more than 300-fold increase of HGT rates (Beaber *et al.*, 2004). Thus the consequences of using SOS response-inducing antibiotics may result in co-selection of other antibiotic resistance genes that are physically linked in a mobile genetic element (Hastings *et al.*, 2004). Moreover, the SOS response controls integron recombination thus enhancing the potential for cassette swapping and capture in cells (Guerin *et al.*, 2009). Besides the antibiotic- and SOS response-regulated excision, transfer and integration of ICEs, regulatory factors include the stationary phase of growth and the presence of 3-chlorobenzoate (Sentchilo *et al.*, 2003). Interestingly, this chlorinated compound stimulates horizontal transfer of the genes encoding its own metabolism. This may explain the previous results of the authors that suggested the need of specific substrates for genetic transfer to occur in activated-sludge microcosms (Ravatn *et al.*, 1998). The transfer of another class of MGEs, GTA, can also be induced by mitomycin C and oxidative stress (Stanton *et al.*, 2008).

Although it has been initially assumed that the SOS response is triggered exclusively by direct DNA damage, there is evidence that certain stimuli can indirectly generate SOS-inducing signals (Aertsen and Michiels, 2006). Some antibiotics, which do not directly interfere with DNA metabolism, may, nevertheless, induce a genuine SOS response. This was demonstrated, for example, for the β-lactam antibiotics targeting bacterial cell wall (Miller *et al.*, 2004). The resulting effect is the elevated rate of horizontal transfer of virulence genes in *staphylococci* (Maiques *et al.*, 2006; Ubeda *et al.*, 2006). Furthermore, the presence of a functional SOS response system seems not an absolute prerequisite for the stress-induced increase in HGT frequencies. In naturally competent bacterial species such as *Streptococcus pneumonia* the antibiotic-imposed stress induces genetic transformation in the absence of a SOS-like system (Prudhomme *et al.*, 2006). Another naturally competent

bacterium, *Legionella pneumophila*, also lacks a prototypic SOS response system, but UV light, which represents a major source of genotoxic stress in the environment, effectively induces competence development in this bacterium (Charpentier *et al.*, 2011). The authors have hypothesized that competence for natural transformation and, therefore, the ability to acquire and propagate foreign genes may have evolved as a DNA damage response in SOS-deficient bacteria. Thus, Gram-positive bacteria respond to stress conditions by induction of competence for genetic transformation to generate genetic diversity (Claverys *et al.*, 2006). The strategy is combined with SOS induction in bacteria, such as *B. subtilis,* while others, such as *S. pneumoniae*, rely solely on competence.

In naturally competent Gram-negative bacteria, the stress conditions may also enhance HGT. *V. cholerae,* for example, can acquire new genetic material by natural transformation during growth on chitin, which activates regulatory cascades leading to the increased cell density, nutrient limitation, and decline in growth rate and stress (Meibom *et al.*, 2005). Stress in the form of DNA damage induces transcription and translation of competence genes in *Helicobacter pylori*, thus increasing transformation frequency and genetic exchange rates (Dorer *et al.*, 2010).

To what extent is the SOS response induced in natural ecosystems? The impact of solar UV radiation on microbiota may be profound, especially in aquatic ecosystems (Hader, 2000; Sinha and Hader, 2002). One of the major targets is DNA where UV-B irradiation results in the formation of cyclobutane-pyrimidine dimers (CPDs), 6-4 photoproducts (6-4PPs), and DNA strand breaks, thus leading to the induction of a number of repair mechanisms, including the SOS response (Rastogi *et al.*, 2010). In photosynthetic cyanobacteria, UV-B stress is accompanied by additional stresses such as oxidative stress and oxidative damage (He *et al.*, 2002). Despite the fact that future trends for solar UV irradiation of the Earth's surface remain unclear, the increases in UV-B irradiance over the latter part of the 20th century have been larger than the natural variability (McKenzie *et al.*, 2007). Thus, this factor may play an increasingly important role in accelerating HGT in microbial ecosystems.

Another important factor that may induce the SOS response in environmental bacteria is the release of DNA-damaging compounds as a result of human activities. Hospitals, for example, represent an indisputable release source of many chemicals compounds in their wastewaters, with a significant genotoxic impact (Jolibois *et al.*, 2003; Jolibois and Guerbet, 2006, Gupta *et al.*, 2009). Genotoxic effects are also associated with wastewater of industrial and domestic origin (Jolibois and Guerbet, 2005; Isidori *et al.*, 2007). Then the release of the genotoxic wastewater into the environment, for example in the form of land application, may lead to the elevated genotoxicity in the corresponding ecosystems (Aleem and Malik, 2003; Alam *et al.*, 2009). In general, soil in highly industrialized areas displays elevated genotoxic effects (Xiao *et al.*, 2006). Genotoxicity or mutagenicity of environmental compounds is usually studied in regards to human and animal health but the techniques used to measure these effects such as SOS chromotest, umu test, Ames mutates and others are mostly based on bacterial systems. Thus, these measurements indirectly benefit our understanding of how the environmental compounds may affect HGT in environmental microbiota.

Antimicrobials

In recent years the concept of antibiotics as solely killing agents has been substantially reassessed in the light of new findings suggesting that low concentrations of antibiotics may play

a regulatory function in natural ecosystems (Davies *et al.*, 2006; Fajardo and Martinez, 2008; Aminov, 2009). Moreover, the killing/therapeutic and regulatory/subinhibitory concentrations of an antibiotic are indeed directed towards different targets in the cell (Hoffman *et al.*, 2005). There is a substantial body of evidence suggesting that the subinhibitory concentrations of antibiotics may significantly increase the frequency of horizontal transfer of many types of mobile genetic elements. It was noticed a quarter of a century ago that the subinhibitory concentrations of β-lactams enhanced the transfer of tetracycline resistance plasmids in *Staphylococcus aureus* by up to 1000-fold (Barr *et al.*, 1986). Pre-incubation of donor cells of *Bacteroides* sp. in the presence of subinhibitory tetracycline accelerates the mobilization of a resident non-conjugative plasmid by chromosomally encoded tetracycline conjugal elements (Valentine *et al.*, 1988). A similar type of exposure of donor *Bacteroides* cells appeared to be a pre-requisite for the excision and conjugal transfer of the ICE CTnDOT (Stevens *et al.*, 1993; Whittle *et al.*, 2002). In the absence of tetracycline induction of donor cells, practically no such transfers were detected. Addition of subinhibitory tetracycline into the mating medium substantially enhanced the conjugal transfer of another ICE, *Tn*916 (Showsh and Andrews, 1992). A similar stimulatory effect of tetracycline on conjugation transfer was demonstrated for *Tn*925 as well (Torres *et al.*, 1991). A recent study has revealed the induction effect of carbadox and metronidazole on the GTA-mediated HGT in *Brachyspira hyodysenteriae* (Stanton *et al.*, 2008). The induced VSH-1 particles transmitted tylosin and chloramphenicol resistances between *B. hyodysenteriae* strains.

These experiments described above have been performed under laboratory conditions using standard mating techniques, and the question is whether these observations reflect the real *in vivo* situation or these are the effects of specific *in vitro* conditions. The former point of view is supported by a number of experiments using animal models. For instance, the inclusion of subinhibitory tetracycline in drinking water resulted in a 10-fold increase of transfer of an ICE, *Tn*1545, from *Enterococcus faecalis* to *Listeria monocytogenes* in the intestine of gnotobiotic mice (Doucet-Populaire *et al.*, 1991). In gnotobiotic rats, the presence of tetracycline resulted in a higher number of *Tn*916 transconjugants compared to control (Bahl *et al.*, 2004). An astonishing 100% transfer rate of a small plasmid pLFE1 from *Lactobacillus plantarum* to *E. faecalis* was observed in the gut of gnotobiotic mice receiving erythromycin (Feld *et al.*, 2008). These experiments suggest that the stimulatory effect of subinhibitory antibiotics on transfer of MGEs is not an artefact of *in vitro* conditions but happens in the real gut of real animals. It needs to be noted, however, that the transfer frequencies were estimated in gnotobiotic animals, which are lacking the highly diverse and dense microbiota in the gut.

The mechanisms contributing to the enhanced movement of MGEs in the presence of antibiotics have been established on several occasions, especially for ICEs. In Tn*916*, transcriptional regulation of the *tra* genes required for the conjugal transfer of this ICE is under the control of *orf7* and *orf8* products (Celli and Trieu-Cuot, 1998; Roberts and Mullany, 2009). Transcripts for these two ORFs are produced from the distant promoter of the *tet*(M) gene as well as from the promoter directly upstream of *orf7*. In the absence of tetracycline, transcription from P_{tetM} is attenuated (Su *et al.*, 1992), and the transcripts are very short, not covering the *orf7* and *orf8* genes. In addition, the product of *orf9* negatively regulates the promoter upstream of *orf7*. In the presence of tetracycline, however, transcription from P_{tetM} extends through *orf7* and *orf8* thus allowing the synthesis of these two proteins that

promote transcription from P_{orf7}. The long transcripts from P_{tetM} are also complementary to the *orf9* transcripts thus efficiently reducing the concentration of P_{orf7} repressor. As a result, transcription from P_{orf7} extends downstream of *orf8* through *int* and *xis*, which encode the integrase and excisionase, respectively. Once Tn*917* is circularized, transcription from P_{orf7} also leads to the expression of the *tra* genes thus initiating the conjugation machinery.

Another mechanism, also involving tetracycline as a positive regulator of horizontal gene transfer, is implemented in the *Bacteroides* ICE, CTnDOT (Whittle *et al.*, 2002; Moon *et al.*, 2005). In this regulatory cascade, tetracycline activates the transcription of the *tet*(Q) gene and the downstream genes of the two-component regulatory system, *rteA* and *rteB*. The product of *rteB* activates the transcription of *rteC* system, which, in turn, leads to the elevated transcription of the gene cluster involved in CTnDOT excision. Subsequently, the proteins encoded by the excision genes upregulate the production of *tra* gene mRNA thus activating the conjugation machinery (Jeters *et al.*, 2009). In this complex regulatory system, the stimulatory effect of tetracycline on transcription of its own resistance gene (and concomitantly on the excision and conjugal transfer genes of CTnDOT) is implemented through a translation attenuation mechanism involving the leader region of *tet*(Q) (Wang *et al.*, 2005).

Both regulatory mechanisms of the MGE movement discussed above display a common theme in that the primary switch for this genetic process is based on an antibiotic-antibiotic resistance gene pair. That is, the presence of a subinhibitory antibiotic in the environment activates the transcription of a corresponding resistance gene and, concurrently, the genes involved in the mobility of MGEs. In this regard, the interaction of an antibiotic and antibiotic resistance gene resembles a positively regulated switch, with an antibiotic possessing a signalling function, ultimately leading to the activation of horizontal gene exchange in microbiota. The recent works describing concentration-dependent bacterial responses to antibiotics have led to the development of the hormesis concept (Davies *et al.*, 2006; Fajardo and Martinez, 2008). According to this concept, low concentrations of antibiotics may regulate a specific set of genes in target bacteria, while increasingly higher concentrations elicit a stress response, and even higher concentrations are lethal. It has been suggested that antibiotics play a regulatory role in nature at low concentrations unlike the lethal concentrations used in clinical therapy (Aminov, 2009). Given the profound stimulatory effect of low-dose antibiotics on the movement of MGEs, one of the functions of antibiotics in natural ecosystems may be the regulation of HGT between the representatives of environmental microbiota.

Despite the fact that some countries have enacted legislations limiting the non-therapeutic use of antibiotics, in particularly in food animals (http://ec.europa.eu/food/food/animalnutrition/feedadditives/index_en.htm), the use of subtherapeutic antimicrobials in agriculture and aquaculture of other countries is still widespread. Moreover, the US Food and Drug Administration has recently approved subinhibitory concentrations of doxycycline and minocycline for the systemic treatment of skin infections in humans (Del Rosso, 2007). Thus, there is a high probability that the gut ecosystems may continue to be hotspots of horizontal gene exchange involving the resident and transient gut microbiota. The land application of manure with residual antibiotics and antibiotic resistance genes of the gut content may further contribute to the enhanced HGT in the environment (Chee-Sanford *et al.*, 2009).

Quorum sensing

The quorum sensing (QS) systems are widespread among a variety of microbiota and initially they were recognized as population-density-sensing mechanisms based on the best-studied prototype, the QS network, which regulates the *lux* operon in *Vibrio fischeri* (Eberhard *et al.*, 1981; Fuqua *et al.*, 1996). Since then it has become clear that the QS is involved in regulation of a much broader range of functions and activities such as pathogenicity, extracellular enzyme production, antibiotic biosynthesis, and others (Bainton *et al.*, 1992; Jones *et al.*, 1993; Passador *et al.*, 1993; Pirhonen *et al.*, 1993; Pierson *et al.*, 1994). The QS is indeed a universal language of communication not only among the bacteria but also in the inter-kingdom interaction (Shiner *et al.*, 2005).

One of the earliest indications of the QS involvement in HGT came from the studies of conjugal transfer of the *Agrobacterium tumefaciens* Ti plasmids (Zhang and Kerr, 1991; Piper *et al.*, 1993; Fuqua and Winans, 1994). The Ti plasmid encodes a regulatory system, consisting of the acylhomoserine lactone (AHL) synthase TraI and the transcription factor TraR. TraI synthesizes AHLs, mainly N-(3-oxo-octanoyl)-L-homoserine lactone (OOHL), while TraR is an OOHL-dependent transcription activator of conjugative transfer genes. Molecular mechanisms of this activation that leads to the enhanced conjugal transfer of the Ti plasmids are well understood (Costa *et al.*, 2009; Quin *et al.*, 2009).

Another well-explored area is the QS regulation of transfer of large symbiotic plasmids in rhizobia (Danino *et al.*, 2003; He *et al.*, 2003; Tun-Garrido *et al.*, 2003). Analysis of plasmid transfer in several rhizobia species has revealed a regulatory relay that is specifically poised to detect AHLs made by different cells and to respond to these signals by up-regulation of conjugal transfer genes. In turn, the production of AHLs, N-(3-oxo-octanoyl)-L-homoserine lactone and N-(octanoyl)-L-homoserine lactone, is regulated by a complex interaction of plasmid- and chromosome-encoded genes in donors and recipients in response to environmental cues. Recently it has been shown that the transfer of an ICE of *Mesorhizobium loti*, which carries genes for a nitrogen–fixing symbiosis with *Lotus* species, is also regulated by a QS mechanism (Ramsay *et al.*, 2009). The two conserved hypothetical genes, which are essential for the QS-mediated excision and transfer, can also be found on putative ICEs in several alpha-proteobacteria, indicating a broader presence of this HGT regulatory mechanism.

Recent investigations have identified an important role played by QS in regulation of phage-mediated HGT. For example, acyl-homoserine lactones (AHLs), the essential signalling molecules of QS in many Gram-negative bacteria, can trigger phage production in soil and groundwater bacteria (Ghosh *et al.*, 2009). Interestingly, in the *recA* mutant of *E. coli* the induction responses of lambda to AHL remained unaffected, suggesting that this mechanism does not involve a SOS response.

Genetic mechanisms involved in maintenance and dissemination of MGEs

The 'cooperation' between different mechanisms of HGT can be seen in many examples of mobility among the genetic elements that are normally not mobile on their own. For example, ICEs of *Bacteroides* spp. and large broad-host-range conjugative IncP plasmids R751 and RP4 are able to act *in trans* to excise, circularize, and transfer unlinked integrated elements called NBUs (non-replicating *Bacteroides* units) (Li *et al.*, 1993; Shoemaker *et al.*, 1993). Bioinformatics of plasmid mobility suggests that globally about a quarter of all plasmids

are potentially mobilizable and, therefore, can be transferred if a compatible conjugation machinery is present (Smillie *et al.*, 2010). In *Sinorhizobium meliloti*, conjugation functions for a 1683 kb symbiotic megaplasmid pSymB are supplied *in trans* by another megaplasmid, pSymB (Blanca-Ordóñez *et al.*, 2010). The recently described IS*CR* elements are thought to be the key players in IncA/C plasmid evolution serving as antibiotic resistance gene capture and movement systems that are also capable of constructing extended clusters of antibiotic resistance genes (Toleman and Walsh, 2010).

Insertion sequences (ISs) constitute an important component of most bacterial genomes and are simple transposable elements consisting of inverted repeat (IR) sequences, a transposase gene, and frequently a second recombination regulation enzyme gene (Mahillon and Chandler, 1998). It has been thought that the transposition of ISs is a rare event within a bacterial genome, but the discovery of a protein called IS-excision enhancer (IEE), which promotes excision events, suggested that the rates could be high (Kusumoto *et al.*, 2011). The IEE activity, therefore, may play an important role in bacterial genome evolution by inducing IS removal and genomic deletion. Another aspect is the transposition of an IS into a conjugative plasmid, an ICE, or a genomic island. This way, the IS elements can be disseminated to many other, not necessarily closely related, bacterial taxa. Together with prophages (Asadulghani *et al.*, 2009), IS elements are the major contributors to the genomic diversification in pathogenic *E. coli* (Ooka *et al.*, 2009). Since the IS elements are ubiquitous in many bacteria, including environmental and commensal species, these mechanisms of diversification are probably not limited exclusively to pathogens.

Since the carriage of MGEs may be associated with a considerable fitness cost, certain mechanisms of stable inheritance have been selected within the mobile genetic elements. One of these mechanisms is a toxin–antitoxin (TA) system consisting of two components, a stable toxin and its labile antitoxin. Type I and II TA systems were found on plasmids in the 1980s (Ogura and Higara, 1983; Jaffe *et al.*, 1985), and type III is a more recent discovery (Fineran *et al.*, 2009). The general mechanism of action is that if an MGE with TA system is lost during cell division, the concentrations of a labile antitoxin rapidly decreases thus releasing a stable toxin, which kills an MGE-free cell (Van Melderen, 2010). Thus, TA systems contribute to the stable maintenance and dissemination of plasmids and genomic islands in bacterial populations despite the associated fitness cost.

The size of MGEs varies from small IS elements (typically 700 to 2500 bp) (Mahillon and Chandler, 1998) to large symbiotic megaplasmids of *Sinorhizobium meliloti* (1.35 Mb and 1.68 Mb) (Barnett *et al.*, 2001; Capela *et al.*, 2001). The majority of large self-transmissible genetic elements have a sufficient capacity to carry multiple genes, including those encoding antibiotic, heavy metal and biocide resistances, metabolism of various substrates and xenobiotics, symbiosis with the host, and other auxiliary functions. The fact that R plasmids mediate resistance to mercury, nickel and cobalt was first described more than 40 years ago (Smith, 1967). Subsequent research by other groups demonstrated that the genetic linkage between antibiotic resistance and mercury resistance in enterobacteria had occurred prior to the late 1950s in Japan (reviewed in Liebert *et al.*, 1999). At the same time, Enterobacteriaceae strains collected by E.D.G. Murray from 1917 to 1954 contained very few antibiotic and mercury resistant bacteria, despite the finding that 25% of the strains carried conjugative functions (Hughes and Datta, 1983). Thus, the frequency of MGEs even in the 'pre-antibiotic era' was sufficiently high but not associated with the adaptation to anthropogenic factors such as antibiotics or heavy metals.

The exceptional natural genetic engineering capabilities of bacteria have been profoundly demonstrated during the 'antibiotic era' (Aminov, 2010). To withstand the massive pressure of antimicrobials used by humans, commensal and pathogenic bacteria were able, within a relatively short period of time on the evolutionary scale, to mobilize a huge reservoir of antibiotic resistance genes, often from the environmental bacteria (Aminov and Mackie, 2007; Cantón, 2009; Wright, 2010). The main genetic engineering tool used by bacteria to collect antibiotic resistance genes is integrons, the genetic platform that is involved in the acquisition and functional expression of exogenous gene cassettes (Mazel, 2006). The discovery of superintegrons that contain hundreds of auxiliary genes and may occupy a significant part of many bacterial genomes has changed our initial interpretation of integrons as merely a mechanism of collecting antibiotic resistance genes. The presence of toxin/antitoxin cassettes in superintegrons further facilitates the stabilization of large cassette arrays consisting of many ancillary genes (Cambray *et al.*, 2010).

The physical linkage of numerous and functionally diverse groups of genes within the high-capacity MGEs has implications for their persistence in the environment. Selection that is imposed towards even a single component/gene of an MGE will automatically select for the whole MGE. Thus, we should see the co-selection effect in phenotypes of bacteria that carry MGEs. Indeed, bacteria in metal-contaminated areas appeared to be more tolerant to metals and antibiotics than in control sites (Stepanauskas *et al.*, 2005; Baker-Austin *et al.*, 2006; Wright *et al.*, 2006). Treatment of agricultural soils with copper may lead to a significantly higher incidence of antibiotic resistance phenotypes in indigenous soil microbiota (Berg *et al.*, 2005). In freshwater microcosms, amendment with metal concentrations representative of industry and mining-impacted environments increased the frequency of antibiotic resistance in the microbial communities (Stepanauskas *et al.*, 2006). Genetic mechanisms responsible for the co-selection phenomena in the contaminated environments are presently poorly understood, but it is clear that the MGE-driven HGT is the main adaptive trait in bacteria inhabiting industrially contaminated aquatic (Wright *et al.*, 2008) and soil (Top *et al.*, 1995; Sobecky and Coombs, 2009) ecosystems.

On the other side of the spectrum are the negative regulators of HGT, genetic mechanisms that constrain gene transfer events. Some of the best-studied systems are receptor masking, restriction-modification and abortive infection that protect the cell from foreign DNA (Hyman and Abedon, 2010; Labrie *et al.*, 2010). This is especially important if it is a bacteriophage DNA that may enter into the lytic cycle and thus destroy the bacterial host. The previously discussed stress and SOS responses are involved into the regulation of restriction-modification activities. Alleviation of EcoK DNA restriction in *E. coli* by UV irradiation and expression of the SOS response, for example, results in a 10^3- to 10^4-fold increase in phage infection and in a 4-fold increase in plasmid transformation rates (Hiom and Sedgwick, 1992). All four resident restriction systems of *E. coli* K-12 show reduced activity following UV treatment (Kelleher and Raleigh, 1994). Thus the previously discussed effects of the SOS response induction also involve the suppression of restriction activity leading to the increased rate of phage infection and natural transformation.

Clustered regularly interspaced short palindromic repeats (CRISPRs) in the genomic sequences of Bacteria and Archaea were discovered during *in silico* analyses almost a decade ago (Jansen *et al.*, 2002). These are direct repeats, varying in size from 21 to 50 bp, interspaced by similarly sized non-repetitive sequences. Initially, four CRISPR-associated (cas) genes were identified in CRISPR-containing prokaryotes that were absent from

CRISPR-negative prokaryotes. Further analyses showed that up to 45 CRISPR-associated (Cas) protein families are uniformly associated with CRISPRs and located near a repeat cluster (Haft *et al.*, 2005). The biological role of CRISPRs remained elusive until 2007, when the experimental evidence suggested that these elements provide acquired resistance against viruses (Barrangou *et al.*, 2007). Following the viral challenge, bacteria integrate new spacers derived from the phage genome and resistance specificity is determined by spacer-phage sequence similarity. Further investigations revealed that this CRISPR/Cas bacterial immune system cleaves invading bacteriophage and plasmid DNA in a manner similar to the RNA interference mechanism in eukaryotes (Garneau *et al.*, 2010).

Very little is known about the regulation of CRISPR/Cas system. In *E. coli* K12 transcription from the casA and CRISPR I promoters is repressed by a heat-stable nucleoid-structuring protein (H-NS) (Pul *et al.*, 2010), which is a global transcription repressor in Gram-negative bacteria. This repression can be relieved by a number of regulators, in particular by LeuO (Cen and Wu, 2005, Westra *et al.*, 2010), a member of the LysR family of transcription factors in Gram-negative enteric bacteria (Stoebel *et al.*, 2008). In *Salmonella enterica* serovar Typhi the CRISPR/Cas system appeared to be regulated similarly, by the antagonistic activities of LeuO and H-NS (Medina-Aparicio *et al.*, 2011). In general, however, the CRISPR/Cas system in wild-type *E. coli* is repressed under laboratory conditions and it does not provide efficient protection against phages (Westra *et al.*, 2010). The involvement of a broader range of model organisms as well as exploration of other cellular regulatory mechanisms may provide a clue on how the CRISPR-based immunity is regulated by environmental factors.

Concluding remarks

It is hypothesized that the extent of horizontal gene exchange in the early stages of life on the Earth was much higher, dominating the evolutionary landscape (Woese, 2000). Only after evolving into more integrated and stable cellular entities the true organismal lineages in the Darwinian sense (e.g. evolving by vertical evolution that is enacted through the natural selection of the fittest among genetically variable individuals) could exist. But, even after the establishment of the three domains of life, HGT continued to play an important role in evolution. The possibility of lateral acquisition of a gene or a group of genes that may confer immediate selective advantage substantially accelerates the evolutionary process; the situation with rapid dissemination of antibiotic resistance serves as a perfect example of this scenario.

It is evident from the retrospective and prospective studies that the rate of horizontal gene exchange in the microbial world is high indeed. The remnants of the past horizontal gene acquisition can be clearly seen in all microbial genomes sequenced to date. It is a sort of ironic situation to realize that many phenotypic characteristics used in microbial taxonomy for years in defining the identity of species, such as the range of substrates utilized, were actually acquired from other organisms. The microbial world around us can be seen as a gigantic microbiome, with the continuous flow of genes between its different compartments. This flow is sustained by a variety of sophisticated natural genetic engineering tools, MGEs, which have been selected during the evolution as providing the means for re-shuffling the available genetic material and picking up the best responses possible to cope with the continuously changing environmental challenges. The recent relatively short history of the

'antibiotic era' (Aminov, 2010) demonstrates the ultimate success of this strategy and urges us to rethink our own when interacting with the microbial world.

Continuous discoveries of novel MGEs and mechanisms of HGT, together with the findings of unexpectedly high HGT rates in natural ecosystems, indicate that we are still far from the understanding of the true extent of HGT in nature. The contribution to the better understanding may be envisaged as the combination of retrospective and prospective approaches. On the dry lab side, the history of past HGT events, which is recorded in the wealth of genomic/genomic information, can be more vigorously interrogated on the basis of our knowledge about MGEs and with the help of bioinformatics tools that are able to detect the events consistent with HGT. On the wet lab side, it is the development of *in situ* technologies that are more sensitive, less intrusive and applicable to the field studies. The microcosm experiments should model real environmental situations as close as possible, working with native microbiota, with a lesser dependence on model organisms. These developments may help to elaborate better strategies to deal with the pressing needs such as the emergence of novel infections and opportunistic pathogens as well as antibiotic resistance genes.

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